

Supplementary Material

High-throughput Screening of Antimicrobial Resistance Genes and their Association with Class 1 Integrins in Urban Rivers in Japan

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Table S1 Primers and probes used for conventional qPCR

Target	Primer/probe	Sequence [5'-3']	Reference
16S rRNA gene	Forward primer	341F: CCTACGGGAGGCAGCAG	(Bru et al., 2008)
	Reverse primer	534R: ATTACCGCGGCTGCTGG	
<i>intI1</i>	Forward prime	intI1F165: CGAACGAGTGGCGGAGGGTG	(Gillings et al., 2015)
	Reverse primer	intI1R476: TACCCGAGAGCTTGGCACCCA	
<i>sul1</i>	Forward prime	sul1_F (6)-492: CGCACCGGAAACATCGCTGCAC	(Pei et al., 2006)
	Reverse primer	sul1_R (6)-492: TGAAGTTCCGCCGCAAGGCTCG	
<i>tetA</i>	Forward prime	tetA_F (6)-542: GCTACATCCTGCTTGCCTTC	(Lee et al., 2017)
	Reverse primer	tetA_R (6)-542: CATAGATCGCCGTGAAGAGG	
crAssphage	Forward prime	CPQ056F1: CAGAAGTACAACTCCTAAAAAACGTAGAG	(Stachler et al., 2017)
	Reverse primer	CPQ056R1: GATGACCAATAAACAAGCCATTAGC	
	Probe	CPQ056P1: [FAM]-AATAACGATTTACGTGATGTAAC-[MGB]	

Table S2 Primers used for HT-qPCR

Classification	Gene code	Assay ID	Forward primer [5'-3']	Reverse primer [5'-3']
Housekeeping gene	16S rRNA	AY1	GGGTTGCGCTCGTTGC	ATGGYTGTCTCGTCAGCTCGTG
	aacC2	AY2	ACGGCATTCTCGATTGCTTT	CCGAGCTTCACGTAAGCATTT
Aminoglycoside	aadA 1	AY10	GTTGTGCACGACGACATCATT	GGCTCGAAGATACCTGCAAGAA
	strB	AY24	GCTCGGTCTGTGAGAACAATCT	CAATTTCGGTCGCCTGGTAGT
	aadA2 3	AY331	CAATGACATTCTTGCGGGTATC	GACCTACCAAGGCAACGCTATG
	aadA1 1	AY395	TGTACGGCTCCGCAGTG	CACGGAATGATGTCGTCGTG
	aadA6	AY411	CCATCGAGCGTCATCTGGAA	CCCGTCTGGCCGGATAAC
	blaVEB	AY105	CCCATGCAAAGCGTTATG	GAAAGATTCCCTTTATCTATCTCAGACAA
Beta-lactam	blaOXY	AY108	CGTTCAGGCGGCAGGTT	GCCGCGATATAAGATTTGAGAATT
	cfxA	AY114	TCATTCTCGTTCAAGTTTTCAGA	TGCAGCACCAAGAGGAGATGT
	blaGES	AY125	GCAATGTGCTCAACGTTCAAG	GTGCCTGAGTCAATTCTTTCAAAG
	blaSFO	AY126	CCGCCGCCATCCAGTA	GGGCCGCCAAGATGCT
	blaCTX-M 5	AY134	GCGATAACGTGGCGATGAAT	GTCGAGACGGAACGTTTCGT
	blaCTX-M 8	AY147	CGTCACGCTGTTGTTAGGAA	CGCTCATCAGCACGATAAAG
	blaNDM	AY152	GGCCACACCAGTGACAATATCA	CAGGCAGCCACCAAAAGC
	blaCMY 3	AY429	CTGGCGCATACCTGGATTAC	GCCAGTTCAGCATCTCCCA
	blaOXA51	AY435	CGACCGAGTATGTACCTGCTTC	TCAAGTCCAATACGACGAGCTA
	blaOXY1	AY436	AAAGGTGACCGCATTCGC	CCAGCGTCAGCTTGCG
	blaSHV11	AY438	TTGACCGCTGGGAAACGG	TCCGGTCTTATCGGCGATAAAC
	blaTEM	AY439	CGCCGCATACACTATTCTCAG	GCTTCATTCAGCTCCGGTTC
Multi-drug resistance (MDR)	acrB 1	AY199	AGTCGGTGTTTCGCCGTTAAC	CAAGGAAACGAACGCAATACC
	acrF	AY201	GCGGCCAGGCACAAAA	TACGCTCTTCCCACGGTTTC
	adeA	AY202	CAGTTCGAGCGCCTATTTCTG	CGCCCTGACCGACCAAT
	emrD 1	AY208	CTCAGCAGTATGGTGGTAAGCATT	ACCAGGCGCCGAAGAAC
	qacEA1 1	AY218	TCGCAACATCCGCATTAAAA	ATGGATTTTCAGAACCAGAGAAAGAAA
	qacEA1 3	AY236	GTCGGTGTTGCTTATGCAGTCT	CAACCAGGCAATGGCTGTAA
	qacF/H	AY489	CTGAAGTCTAGCCATGGATTCACTAG	CAAGCAATAGCTGCCACAAGC
	pcoA	AY495	TGGCGTATGGAGTTTCAATGC	GAATAATGCCGTGCCAGTGAA
MLSB	ermF 1	AY46	CAGCTTTGGTTGAACATTTACGAA	AAATTCCTAAAATCACAACCGACAA
	pncA	AY73	GCAATCGAGGCGGTGTTC	TTGCCGAGCCAATTCA
	ereA	AY528	GATAATTCTGCTGGCGCACA	GCAGGCGTGGTCACAAC
	ereA 2	AY529	TCGTATATGGCGGGCGTAGTA	GGTCCAAGATGGGTGAATGCA
	ermB 2	AY533	GAACACTAGGGTTGTTCTTGCA	CTGGAACATCTGTGGTATGGC
	mphA	AY539	TCAGCGGGATGATCGACTG	GAGGGCGTAGAGGGCGTA
	ermX 2	AY546	TGATGACGGCTCAGTGG	GTGCACCAGCGCCTGA
	ermB 3	AY547	TGAAAGCCATGCGTCTGAC	TTCAGCTGGCAGCTTAAGC
	msrE	AY553	CGGCAGATGGTCTGAGCTTAAA	CGCACTCTTCCTGCATAAAGGA

Table S2 Primers used for HT-qPCR (continued)

Classification	Gene	Assay ID	Forward primer [5'-3']	Reverse primer [5'-3']
Phenicol	catB3	AY29	GCACTCGATGCCTTCCAAAA	AGAGCCGATCCAAACGTCAT
	cmlA_2	AY35	TAGGAAGCATCGGAACGTTGAT	CAGACCGAGCACGACTGTTG
	cmxA	AY37	GCGATCGCCATCCTCTGT	TCGACACGGAGCCTTGGT
	cmlA_4	AY41	GCGCTCTTCGAGGATTCG	CCGCCCCAAGCAGAAGTAGAC
Polymyxin	mcr1	AY466	CACATCGACGGCGTATTCTG	CAACGAGCATACCGACATCG
Quinolone	qnrB	AY96	GCGACGTTCAAGTGGTTCAGA	GCTGCTCGCCAGTCGAA
	qnrD	AY459	CGCTGGAATGGCACTGTGA	GCTCTCCATCCAACTTCACTCC
	qnrS2	AY461	TCCCGAGCAAACCTTTGCCAA	GGTGAGTCCCTATCCAGCGA
Sulfonamide	sul1_2	AY245	GCCGATGAGATCAGACGTATTG	CGCATAGCGCTGGGTTTC
	sul2_2	AY365	TCATCTGCCAACTCGTCGTTA	GTCAAAGAACGCCGCAATGT
Tetracycline	tetA_2	AY254	CTCACCAGCCTGACCTCGAT	CACGTTGTTATAGAAGCCGCATAG
	tetQ	AY259	CGCCTCAGAAGTAAGTTCATACACTAAG	TCGTTTCATGCGGATATTATCAGAAT
	tetW	AY263	ATGAACATTCCCACCGTTATCTTT	ATATCGGCGGAGAGCTTATCC
	tetX	AY267	AAATTTGTTACCGACACGGAAGTT	CATAGCTGAAAAAATCCAGGACAGTT
	tetE	AY273	TTGGCGCTGTATGCAATGAT	CGACGACCTATGCGATCTGA
	tetM	AY574	GGAGCGATTACAGAATTAGGAAGC	TCCATATGTCCTGGCGTGTC
	tetR	AY577	CCGTCAATGCGCTGATGAC	GCCAATCCATCGACAATCACC
Trimethoprim	dfrA1_1	AY284	GGAATGGCCCTGATATTCCA	AGTCTTGCGTCCAACCAACAG
	dfrA1	AY579	CGGATCATGTCAATTGTTTCAGG	ATGTTAGAGGCGAAGTCTTGG
	dfrA17	AY581	CGGGAACGGCCCTGATATTCC	CGTGTTGCGACCGCATACTTTC
	dfrA27	AY586	GCCGCTCAGGATCGGTA	GTCGAGATATGTAGCGTGTCG
MGE	intI1_2	AY289	CGAAGTCGAGGCATTTCTGTC	GCCTTCCAGAAAACCGAGGA
	intI1_1	AY293	CGAACGAGTGGCGGAGGGTG	TACCCGAGAGCTTGGCACCCA
	Tp614	AY297	GGAAATCAACGGCATCCAGTT	CATCCATGCGCTTTTGTCTCT
	tnpA_1	AY299	GCCGCACTGTCGATTTTTATC	GCGGGATCTGCCACTTCTT
	tnpA_2	AY300	CCGATCACGGAAGCTCAAG	GGCTCGCATGACTTCGAATC
	ISPps	AY309	CACACTGCAAAAACGCATCCT	TGTCTTTGGCGTCACAGTTCTC
	IS26_1	AY512	ATGGATGAAACCTACGTGAAGGTC	CGGTACTTAATCTGTGGTGTTC
	ISCR1	AY519	ATGGTTTCATGCGGGTT	CTGAGGGTGTGAGCGAG
	Tn3	AY523	GCTGAGGTGTTTACGCTACATCC	GCTGAGGTAGTCACAGGCATTC
	Tn5403	AY524	AAGCGAATGGCGCGAAC	CGCGCAGGGTAAACTGC

Table S3 Water quality data

River	Code (Group)	pH	Water temp. (°C)	Conductivity (μS/cm)	Ammonium (mg N/L)	Total cell count (cells/mL)	T. coli (CFU/100 mL)	<i>E. coli</i> (CFU/100 mL)
Tamagawa River	TM1 (A)	7.4	19.4	81	0.034	3.3×10^6	1.4×10^3	$< 3.3 \times 10^1$
	TM2 (A)	7.6	22.9	137	0.055	2.7×10^6	5.9×10^3	3.3×10^1
	TM3 (C)	7.7	25.2	248	0.041	6.2×10^6	1.2×10^4	5.3×10^2
	TM4 (C)	7.8	29.2	275	0.089	5.0×10^6	1.2×10^4	2.5×10^3
	TM5 (C)	7.9	28.1	264	0.048	6.1×10^6	5.9×10^3	8.3×10^2
Akikawa River	AK (A)	7.4	22.6	118	0.038	1.8×10^6	8.3×10^3	1.0×10^2
Yaji River	YJ (A)	7.8	24.1	195	0.067	5.1×10^6	1.1×10^4	4.0×10^2
Kitaasa River	KA (A)	7.4	20.1	148	0.049	5.8×10^5	5.4×10^3	$< 3.3 \times 10^1$
Minamiasa River	MA (A)	7.5	22.1	128	0.055	9.4×10^5	6.4×10^3	6.7×10^1
Asakawa River	AS (C)	7.8	26.2	192	0.060	1.1×10^6	5.6×10^3	2.0×10^2
Nogawa River	NO (A)	8.6	30.3	241	0.082	4.5×10^6	1.9×10^4	1.0×10^3
Iruma River	IR1 (A)	7.8	17.2	103	0.010	1.6×10^5	$< 3.3 \times 10^1$	$< 3.3 \times 10^1$
	IR2 (B)	7.6	25.6	203	0.150	3.4×10^6	6.4×10^3	2.7×10^2
Arakawa River	AR1 (B)	7.6	12	252	0.151	4.1×10^6	1.0×10^4	9.0×10^2
	AR2 (C)	6.8	16.9	407	NA	1.9×10^7	1.3×10^4	8.7×10^2
Hokota River	HO (A)	7.3	10.8	355	0.212	2.1×10^6	2.6×10^4	6.7×10^2
Koise River	KI (B)	7.3	10.1	202	0.135	2.6×10^6	1.6×10^4	1.7×10^2
Sakura River	SK (A)	7.2	11.2	209	0.074	3.1×10^6	5.8×10^3	3.3×10^2
Kokai River	KK (B)	7.2	12.7	206	0.048	3.3×10^6	2.4×10^3	3.3×10^1
Kinugawa River	KN (B)	7.4	11.2	190	0.184	3.3×10^6	4.0×10^3	3.3×10^1
Ohori River	OH (A)	7.6	12.1	351	0.187	4.2×10^6	2.7×10^3	6.7×10^1
Edogawa River	ED (A)	7.7	7.2	278	0.124	5.4×10^6	1.6×10^3	$< 3.3 \times 10^1$
Sumida River	SM (C)	7.7	13.7	3999	0.051	8.4×10^6	1.8×10^3	2.0×10^2
Nakagawa River	NK (B)	7.5	12.7	686	1.250	1.7×10^7	1.7×10^3	6.7×10^1
Shinshiba River	SS (B)	7.4	9.9	3999	2.62	8.2×10^6	3.7×10^2	3.3×10^1
Motoara River	MT (C)	7.7	7.1	410	0.525	9.2×10^6	3.7×10^3	2.3×10^2
Sakawa River	SW (B)	7.8	11	193	0.019	2.2×10^6	3.8×10^3	3.3×10^1
Sagami River	SG (A)	7.7	13.8	227	0.022	3.4×10^6	6.7×10^2	1.3×10^2
Sakai River	SI (C)	7.4	16	1725	0.909	2.6×10^7	8.8×10^3	1.1×10^3
Tsurumi River	TR (C)	7.3	15.8	683	0.382	3.3×10^7	3.5×10^3	2.3×10^2

NA: not available

Table S4 Statistical information of nanopore sequencing

River	Code	Total bases (Mb)	Number of raw reads	Mean length of raw reads(bp)
Tamagawa River	TM1	14.0	27,347	511.8
	TM2	17.8	20,038	887.2
	TM3	21.6	31,011	696.9
	TM4	19.9	33,452	596.3
	TM5	18.5	23,674	782.9
Akikawa River	AK	19.4	24,074	805.0
Yaji River	YJ	28.6	40,921	699.4
Kitaasa River	KA	22.7	23,401	969.8
Minamiasa River	MA	29.1	32,997	881.8
Asakawa River	AS	20.7	19,299	1073.4
Nogawa River	NO	19.5	24,945	781.5
Iruma River	IR1	24.3	32,711	743.1
	IR2	23.7	29,415	807.0
Arakawa River	AR1	17.1	20,533	832.9
	AR2	24.2	35,099	690.4
Hokota River	HO	16.3	16,633	982.4
Koise River	KI	23.8	31,559	755.7
Sakura River	SK	18.5	24,124	768.6
Kokai River	KK	22.5	30,198	744.9
Kinugawa River	KN	20.3	21,933	923.5
Ohori River	OH	19.5	22,735	856.2
Edogawa River	ED	12.7	14,818	857.0
Sumida River	SM	14.5	13,827	1050.9
Nakagawa River	NK	18.9	25,990	725.6
Shinshiba River	SS	17.7	18,330	968.3
Motoara River	MT	18.8	24,397	772.4
Sakawa River	SW	18.3	20,331	902.0
Sagami River	SG	20.1	25,802	780.5
Sakai River	SI	18.2	29,539	617.7
Tsurumi River	TR	14.8	24,733	597.0
WWTP A	A-Inf	20.1	23,410	859.3
	A-Eff	19.2	37,495	512.5
WWTP B	B-Inf	24.3	24,602	986.1
	B-Eff	20.3	23,430	864.9
WWTP C	C-Inf	20.0	22,163	900.4
	C-Eff	25.8	39,436	654.0
WWTP D	D-Inf	18.2	16,807	1080.4
	D-Eff	22.0	37,788	580.9

Table S5 Contig sizes of class 1 integron GCs

River	Code	Number of contigs	Min (bp)	1 st quartile (bp)	2 nd quartile (bp)	3 rd quartile (bp)	Max (bp)
Tamagawa River	TM1	870	500	590	883	1095	4580
	TM2	1019	500	867	973	1165	3034
	TM3	1127	516	659	810	1004	2144
	TM4	1128	520	660	975	1043	1836
	TM5	895	503	822	1005	1168	2554
Akikawa River	AK	946	527	646	874	1164	2868
Yaji River	YJ	765	520	984	1418	1604	3379
Kitaasa River	KA	1213	501	571	593	812	4043
Minamiasa River	MA	1132	517	576	647	1085	2090
Asakawa River	AS	702	508	975	1003	1503	2536
Nogawa River	NO	1024	501	659	981	1043	1739
Iruma River	IR1	933	500	642	983	1155	3027
	IR2	777	517	688	1005	1516	2473
Arakawa River	AR1	817	539	659	1037	1512	1915
	AR2	1045	519	804	991	1119	1931
Hokota River	HO	646	526	994	1144	1515	2768
Koise River	KI	571	525	992	1025	1167	1762
Sakura River	SK	714	500	986	1143	1518	2551
Kokai River	KK	983	508	886	1013	1171	2486
Kinugawa River	KN	1004	511	982	1004	1168	2524
Ohori River	OH	736	620	995	1149	1522	2397
Edogawa River	ED	875	544	737	1016	1512	2531
Sumida River	SM	877	515	993	1489	1535	2784
Nakagawa River	NK	1228	557	645	977	1154	1763
Shinshiba River	SS	1017	525	844	884	1168	1763
Motoara River	MT	902	504	659	1031	1508	1764
Sakawa River	SW	802	516	971	1139	1511	2546
Sagami River	SG	873	500	724	989	1043	2491
Sakai River	SI	1068	513	660	835	1034	1859
Tsurumi River	TR	1061	529	680	966	1144	1728
WWTP A	A-Inf	898	511	963	1002	1168	2366
	A-Eff	1048	500	826	999	1168	2503
WWTP B	B-Inf	785	501	959	1001	1161	2365
	B-Eff	646	526	822	1111	1983	2010
WWTP C	C-Inf	819	505	787	1002	1152	1932
	C-Eff	950	509	813	995	1290	2089
WWTP D	D-Inf	764	508	778	997	1156	1931
	D-Eff	1041	504	791	994	1224	1989

Table S6 Classification of class 1 integron GCs containing ARGs

ARGs acquired in class 1 integron GCs	Category	Total number of contigs (% of total contigs containing ARGs)
Class 1 integron GCs carrying one ARG		
<i>bla</i> _{GES-24}	BLA	3519 (15.5%)
<i>aadA2</i>	AMG	3491 (15.4%)
<i>qacH</i>	MDR	3397 (15.0%)
<i>aadA5</i>	AMG	1824 (8.0%)
<i>aadA1</i>	AMG	1778 (7.8%)
<i>aac(6')-31</i>	AMG	1552 (6.8%)
<i>ere(A)</i>	MLSB	1142 (5.0%)
<i>ant(3'')-Ia</i>	AMG	359 (1.6%)
<i>dfrA32</i>	TMP	334 (1.5%)
<i>bla</i> _{GES-3}	BLA	278 (1.2%)
<i>bla</i> _{OXA-21}	BLA	210 (0.9%)
<i>aadA24</i>	AMG	168 (0.7%)
<i>aadA8b</i>	AMG	154 (0.7%)
<i>aadA15</i>	AMG	147 (0.6%)
<i>bla</i> _{OXA-10}	BLA	144 (0.6%)
<i>cmlA1</i>	PHE	119 (0.5%)
<i>aac(6')-IIc</i>	AMG	118 (0.5%)
<i>dfrA5</i>	TMP	109 (0.5%)
<i>dfrA27</i>	TMP	104 (0.5%)
<i>bla</i> _{GES-5}	BLA	93 (0.4%)
<i>aac(6')-Ib</i>	AMG	92 (0.4%)
<i>dfrA17</i>	TMP	78 (0.3%)
<i>aadA10</i>	AMG	70 (0.3%)
<i>aadA2b</i>	AMG	56 (0.2%)
<i>dfrA25</i>	TMP	55 (0.2%)
<i>bla</i> _{CARB-2}	BLA	52 (0.2%)
<i>aadA17</i>	AMG	41 (0.2%)
<i>catB8</i>	PHE	36 (0.2%)
<i>aadA9</i>	AMG	33 (0.1%)
<i>aac(6')-Ib-Hangzhou</i>	AMG	31 (0.1%)
<i>bla</i> _{OXA-2}	BLA	29 (0.1%)
<i>dfrB4</i>	TMP	26 (0.1%)
<i>qacE</i>	MDR	22 (0.1%)
<i>aadA3</i>	AMG	21 (0.1%)
<i>catB3</i>	PHE	16 (0.1%)
<i>aadA11</i>	AMG	12 (0.1%)
<i>dfrB1</i>	TMP	12 (0.1%)
<i>bla</i> _{OXA-4}	BLA	8 (0.0%)
<i>aadA12</i>	AMG	6 (0.0%)
<i>aadA23</i>	AMG	6 (0.0%)

Table S6 Classification of class 1 integron GCs containing ARGs (continued)

ARGs acquired in class 1 integron GCs	Category	Total number of contigs (% of total contigs containing ARGs)
Class 1 integron GCs carrying one ARG		
<i>bla</i> _{OXA-246}	BLA	6 (0.0%)
<i>dfrA33</i>	TMP	6 (0.0%)
<i>bla</i> _{GES-4}	BLA	5 (0.0%)
<i>dfrA14</i>	TMP	5 (0.0%)
<i>aac(6')-Ib-Suzhou</i>	AMG	4 (0.0%)
<i>bla</i> _{IMP-1}	BLA	4 (0.0%)
<i>bla</i> _{OXA-539}	BLA	4 (0.0%)
<i>cml</i>	PHE	4 (0.0%)
<i>aac(6')-II</i>	AMG	3 (0.0%)
<i>aadA4</i>	AMG	3 (0.0%)
<i>aadA6</i>	AMG	3 (0.0%)
<i>aac(6')-IIa</i>	AMG	2 (0.0%)
<i>bla</i> _{CARB-3}	BLA	2 (0.0%)
<i>bla</i> _{GES-6}	BLA	2 (0.0%)
<i>bla</i> _{VEB-1}	BLA	2 (0.0%)
<i>aac(6')-29b</i>	AMG	1 (0.0%)
<i>aadA22</i>	AMG	1 (0.0%)
<i>aadA8</i>	AMG	1 (0.0%)
<i>bla</i> _{IMP-60}	BLA	1 (0.0%)
<i>bla</i> _{OXA-1}	BLA	1 (0.0%)
<i>bla</i> _{OXA-17}	BLA	1 (0.0%)
<i>bla</i> _{OXA-415}	BLA	1 (0.0%)
<i>bla</i> _{VEB-2}	BLA	1 (0.0%)
<i>dfrA12</i>	TMP	1 (0.0%)
<i>dfrA21</i>	TMP	1 (0.0%)
Class 1 integron GCs carrying two ARGs		
<i>aadA2, qacH</i>	AMG, MDR	1568 (6.9%)
<i>bla</i> _{VEB-1} , <i>catB8</i>	BLA, PHE	195 (0.9%)
<i>aac(6')-IIa, ere(A)</i>	AMG, MLSB	177 (0.8%)
<i>aadA2b, bla</i> _{OXA-1}	AMG, BLA	174 (0.8%)
<i>aadA2, cmlA1</i>	AMG, PHE	90 (0.4%)
<i>aac(6')-IIc, catB8</i>	AMG, PHE	84 (0.4%)
<i>aadA5, dfrA17</i>	AMG, TMP	80 (0.4%)
<i>aadA1, catB8</i>	AMG, PHE	39 (0.2%)
<i>aadA8b, qacH</i>	AMG, MDR	27 (0.1%)
<i>aadA2b, bla</i> _{OXA-320}	AMG, BLA	22 (0.1%)
<i>aadA5, qacH</i>	AMG, MDR	22 (0.1%)
<i>aadA6, catB3</i>	AMG, PHE	21 (0.1%)
<i>aadA1, qacH</i>	AMG, MDR	19 (0.1%)

Table S6 Classification of class 1 integron GCs containing ARGs (continued)

ARGs acquired in class 1 integron GCs	Category	Total number of contigs (% of total contigs containing ARGs)
Class 1 integron GCs carrying two ARGs		
<i>aac(6')-31, qacH</i>	AMG, MDR	17 (0.1%)
<i>aac(6')-Ib, aadA1</i>	AMG, AMG	17 (0.1%)
<i>aadA1, catB3</i>	AMG, PHE	15 (0.1%)
<i>bla_{VEB-1}, catB3</i>	BLA, PHE	12 (0.1%)
<i>aar-3, dfrA27</i>	RFM, TMP	10 (0.0%)
<i>dfrA32, ere(A)</i>	TMP, MLSB	9 (0.0%)
<i>aac(6')-31, bla_{OXA-17}</i>	AMG, BLA	6 (0.0%)
<i>aadA2, catB3</i>	AMG, PHE	6 (0.0%)
<i>aadA3, qacH</i>	AMG, MDR	6 (0.0%)
<i>aac(6')-31, catB8</i>	AMG, PHE	5 (0.0%)
<i>aadA2b, bla_{OXA-9}</i>	AMG, BLA	5 (0.0%)
<i>aadA5, bla_{OXA-21}</i>	AMG, BLA	5 (0.0%)
<i>bla_{OXA-21}, catB3</i>	AMG, PHE	5 (0.0%)
<i>aadA2, dfrA12</i>	AMG, TMP	4 (0.0%)
<i>aac(6')-31, aadA2</i>	AMG, AMG	3 (0.0%)
<i>aadA16, catB3</i>	AMG, PHE	3 (0.0%)
<i>aadA2, bla_{GES-5}</i>	AMG, BLA	3 (0.0%)
<i>aadA2, bla_{OXA-129}</i>	AMG, BLA	3 (0.0%)
<i>aadA2, bla_{OXA-9}</i>	AMG, BLA	3 (0.0%)
<i>aadA5, ere(A)</i>	AMG, MLSB	3 (0.0%)
<i>aac(6')-Ib, aadA5</i>	AMG, AMG	2 (0.0%)
<i>aadA1, bla_{OXA-17}</i>	AMG, BLA	2 (0.0%)
<i>aadA1, bla_{OXA-4}</i>	AMG, BLA	2 (0.0%)
<i>aadA2, catB8</i>	AMG, PHE	2 (0.0%)
<i>aadA2, ere(A)</i>	AMG, MLSB	2 (0.0%)
<i>aadA23, catB8</i>	AMG, PHE	2 (0.0%)
<i>aadA2b, qacH</i>	AMG, MDR	2 (0.0%)
<i>aac(6')-Ib, aadA3</i>	AMG, AMG	1 (0.0%)
<i>aac(6')-Ib-Suzhou, bla_{GES-24}</i>	AMG, BLA	1 (0.0%)
<i>aadA1, bla_{OXA-1}</i>	AMG, BLA	1 (0.0%)
<i>aadA1, bla_{VEB-3}</i>	AMG, BLA	1 (0.0%)
<i>aadA10, qacH</i>	AMG, MDR	1 (0.0%)
<i>aadA11, catB3</i>	AMG, PHE	1 (0.0%)
<i>aadA12, qacH</i>	AMG, MDR	1 (0.0%)
<i>aadA15, aadA8b</i>	AMG, AMG	1 (0.0%)
<i>aadA15, bla_{OXA-1}</i>	AMG, BLA	1 (0.0%)
<i>aadA17, catB8</i>	AMG, PHE	1 (0.0%)
<i>aadA17, dfrA12</i>	AMG, TMP	1 (0.0%)
<i>aadA17, qacH</i>	AMG, MDR	1 (0.0%)
<i>aadA2, ant(2'')-Ia</i>	AMG, AMG	1 (0.0%)

Table S6 Classification of class 1 integron GCs containing ARGs (continued)

ARGs acquired in class 1 integron GCs	Category	Total number of contigs (% of total contigs containing ARGs)
<i>aadA2, aar-3</i>	AMG, RFM	1 (0.0%)
<i>aadA2, bla_{OXA-1}</i>	AMG, BLA	1 (0.0%)
<i>aadA2, cml</i>	AMG, PHE	1 (0.0%)
<i>aadA22, qacH</i>	AMG, MDR	1 (0.0%)
<i>aadA23, qacH</i>	AMG, MDR	1 (0.0%)
<i>aadA24, catB8</i>	AMG, PHE	1 (0.0%)
<i>aadA24, dfrA17</i>	AMG, TMP	1 (0.0%)
<i>aadA24, qacH</i>	AMG, MDR	1 (0.0%)
<i>aadA2b, bla_{OXA-246}</i>	AMG, BLA	1 (0.0%)
<i>aadA2b, catB3</i>	AMG, PHE	1 (0.0%)
<i>aadA2b, cmlA1</i>	AMG, PHE	1 (0.0%)
<i>aadA2b, dfrA12</i>	AMG, TMP	1 (0.0%)
<i>aadA3, bla_{OXA-9}</i>	AMG, BLA	1 (0.0%)
<i>aadA3, catB3</i>	AMG, PHE	1 (0.0%)
<i>aadA5, aadA8b</i>	AMG, AMG	1 (0.0%)
<i>aadA5, bla_{IMP-1}</i>	AMG, BLA	1 (0.0%)
<i>aadA8b, bla_{OXA-4}</i>	AMG, BLA	1 (0.0%)
<i>aadA8b, catB8</i>	AMG, PHE	1 (0.0%)
<i>ant(3'')-Ia, qacH</i>	AMG, MDR	1 (0.0%)
<i>bla_{GES-24}, catB8</i>	BLA, PHE	1 (0.0%)
<i>bla_{GES-24}, ere(A)</i>	BLA, MLSB	1 (0.0%)
<i>bla_{VEB-2}, catB8</i>	BLA, PHE	1 (0.0%)
<i>bla_{VEB-4}, catB8</i>	BLA, PHE	1 (0.0%)
Class 1 integron GCs carrying three ARGs		
<i>aac(6')-IIa, bla_{OXA-21}, catB3</i>	AMG, BLA, PHE	172 (0.8%)
<i>aac(6')-IIa, aac(6')-IIa, ere(A)</i>	AMG, AMG, MLSB	9 (0.0%)
<i>aac(6')-Ib, bla_{OXA-129}, catB3</i>	AMG, BLA, PHE	7 (0.0%)
<i>aac(6')-Ib, aac(6')-Ib, bla_{OXA-246}</i>	AMG, AMG, BLA	4 (0.0%)
<i>aac(6')-Ib, aac(6')-Ib, bla_{OXA-10}</i>	AMG, AMG, BLA	2 (0.0%)
<i>aadA1, bla_{VEB-1}, catB8</i>	AMG, BLA, PHE	2 (0.0%)
<i>aac(6')-IIa, aac(6')-IIa, aadA15</i>	AMG, AMG, AMG,	1 (0.0%)

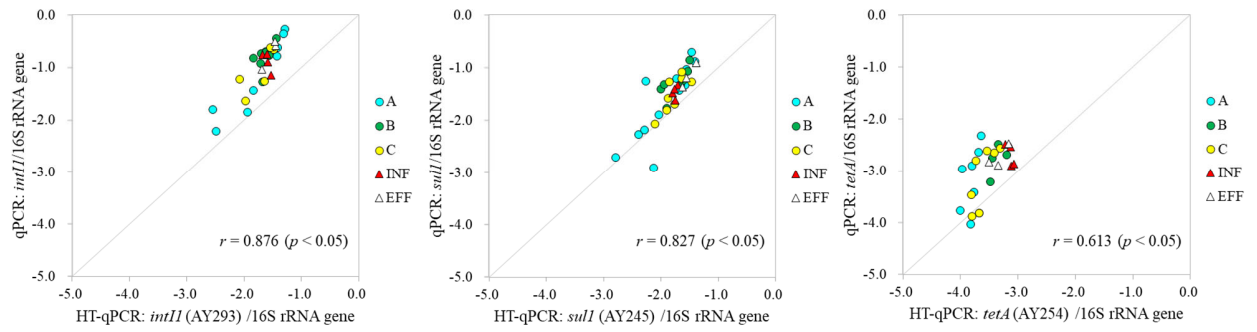


Figure S1 Comparison between HT-qPCR and conventional qPCR. (a) *intI1*/16S rRNA gene, (b) *sulI*/16S rRNA gene, (c) *tetA*/16S rRNA gene.

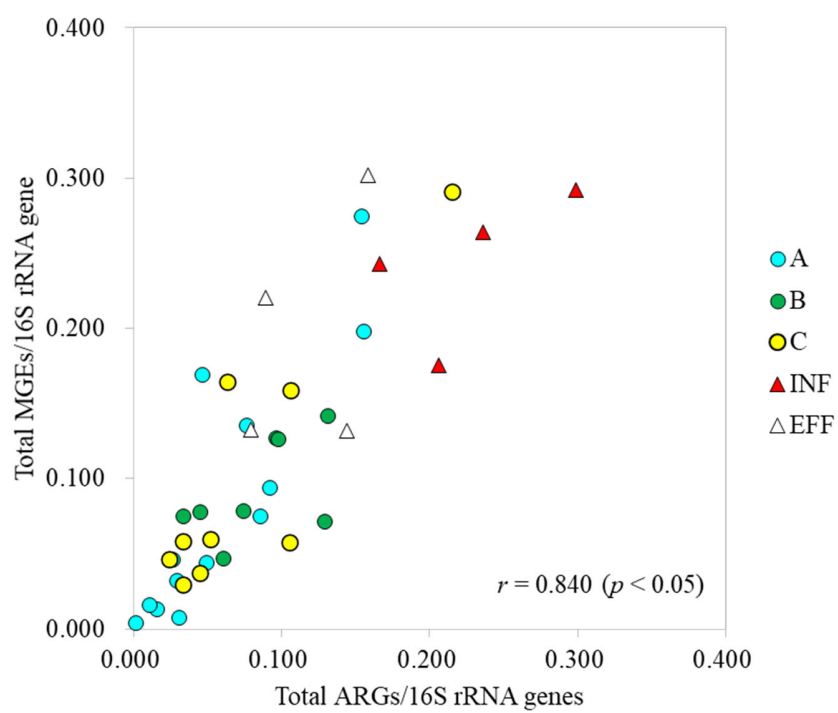


Figure S2 Relationship between relative abundances of total ARGs and total MGEs.

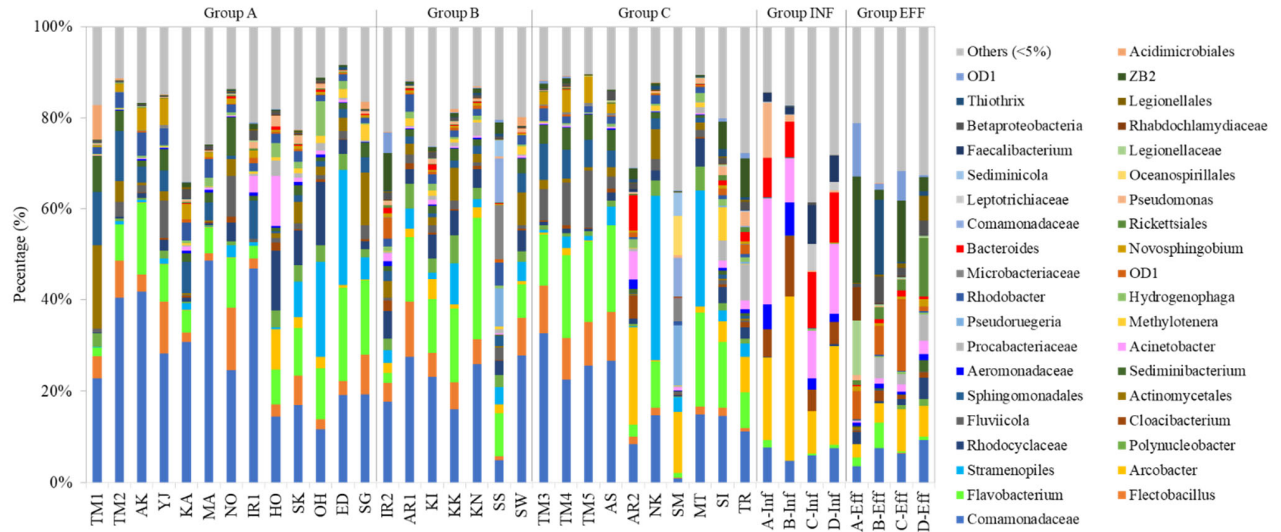


Figure S3 Microbial community structures analyzed by amplicon sequencing of 16S rRNA genes.

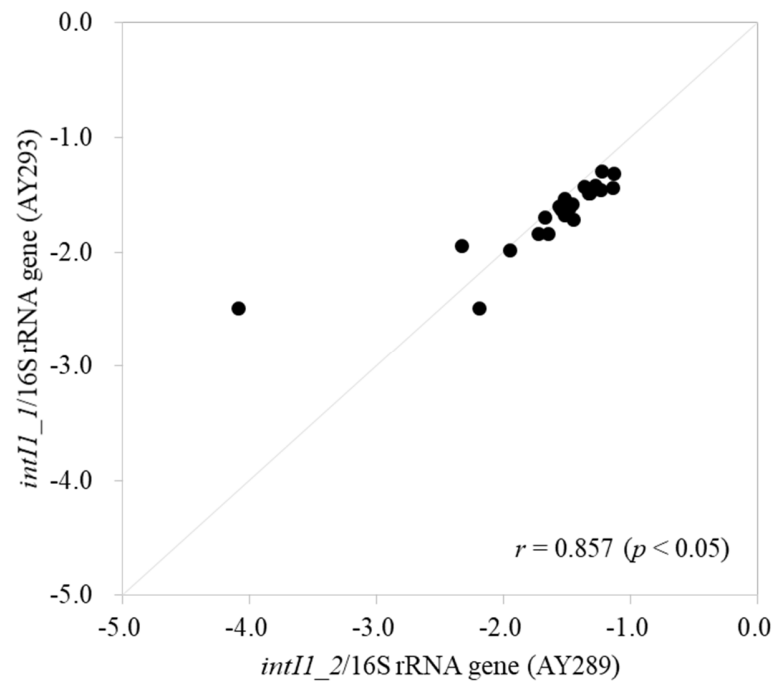


Figure S4 Comparison of relative abundances of *intII* between AY289 and AY293.

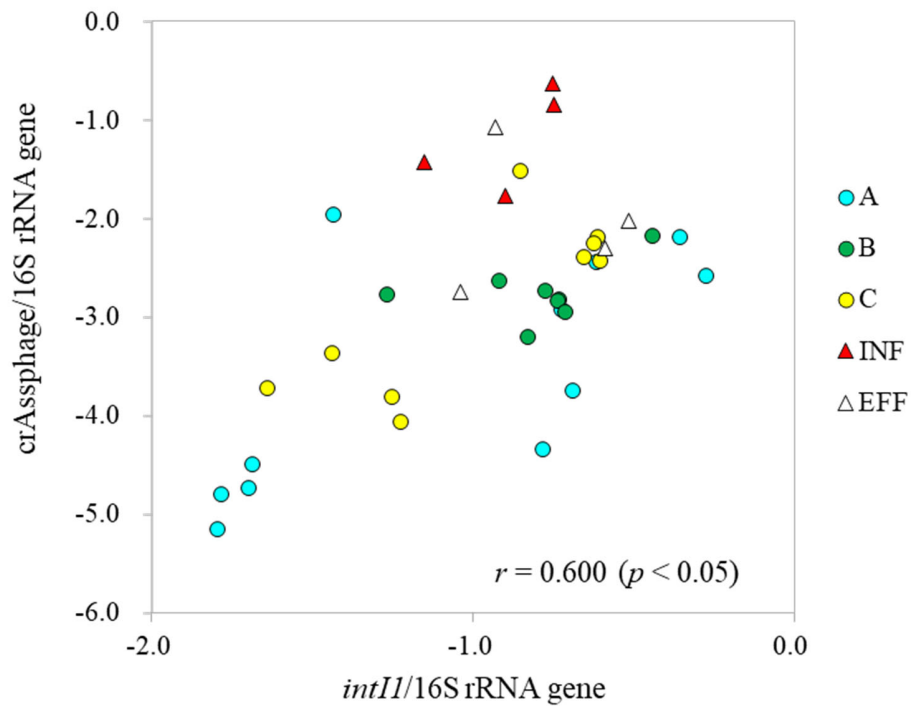


Figure S5 Relative abundances of *int11* and *crAssphage* determined by conventional qPCR.

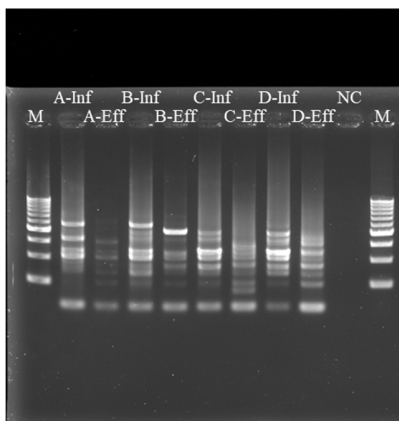
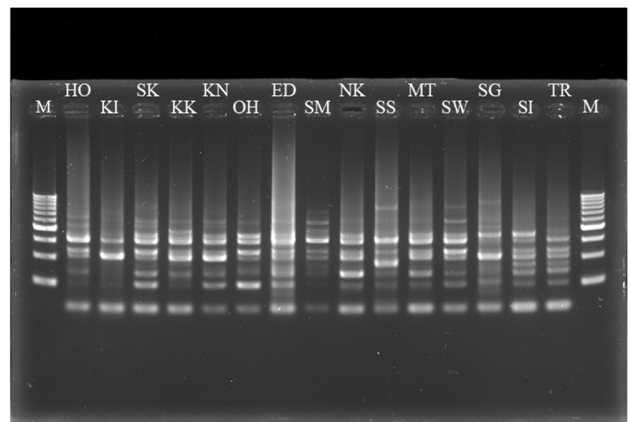
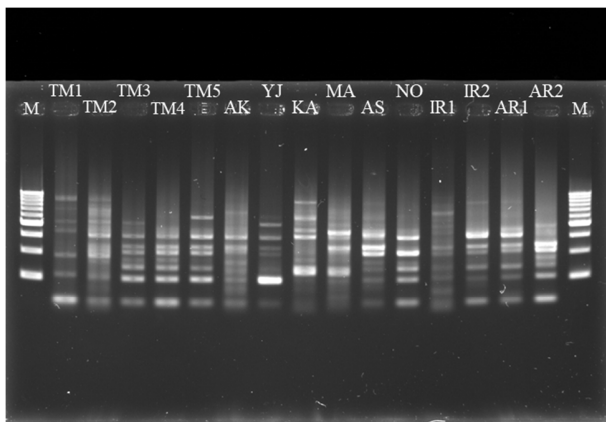


Figure S6 Electrophoresis of class 1 GCs on 1.0% agarose gel. M: 500 bp ladder (500–5,000 bp). NC: negative control.

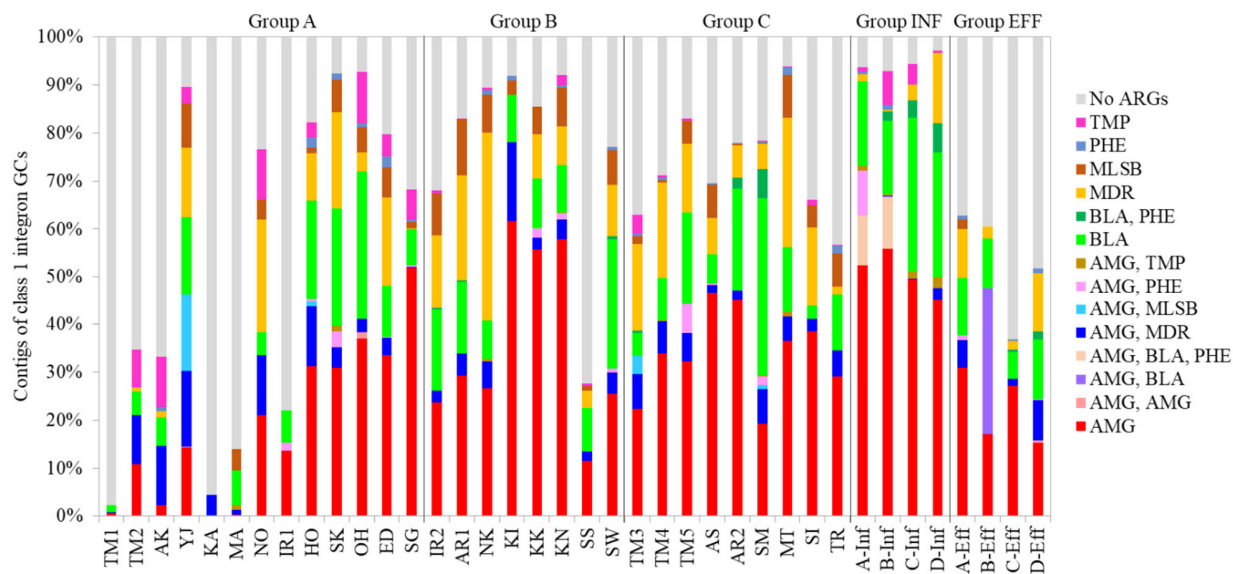


Figure S7 Composition of contigs of class 1 integron GCs.

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